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(AGRICULTURAL RESEARCH SECTION)

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OBSERVATIONS ON THE CATTLE PITUITARY

BY

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Summary.

Observations have been recorded on the weights and measurements of 1,282 cull dairy-cow and beef-steer pituitary glands, and on the weights only of some 225 glands collected from cattle during the course of various experimental procedures. The chief points of interest arising from the investigation are:

1. Characteristic shape differences were seen between dairy-cow and beef-steer pituitaries such that the former are characterized by a higher proportion of round glands and the latter by a higher proportion of pear-shaped glands. Such shape differences appear to be related to the relative proportions of the anterior and posterior lobes, as the evidence showed that dairy cows have larger anterior lobes and smaller posterior lobes than beef steers. The whole pituitary weight for dairy cows was greater than that for beef steers.

2. For sterile cows with cystic ovaries the pituitary weight was found to be significantly greater than that for animals with normal ovaries. A similar weight difference from the normal was shown to occur in glands from cows treated with stilbestrol. Limited treatment with pregnant-mare serum, thiourea, and Thyroprotein had no significant effect on pituitary weight. No significant pituitary weight-difference was observed between pregnant and normal non-pregnant cows.

3. The presence of large "colloid" cysts in the cleft of some 30 per cent of the pituitary glands of approximately 1,500 dairy cows was observed. Only 2 per cent of such cysts occurred in approximately 1,500 beef-steer glands. The nature and cause of these bodies is obscure.

INTRODUCTION.

In comparison with many other species the histology of the cattle pituitary has been little studied, but considerable information has been collected relating the size of this gland to various bodily conditions of the animal. Reece and Turner (1937) and Lewis and Turner (1939) tabulated figures from the available literature both for cattle and for other species, while presenting their own results. Gilmore *et al.* (1941) again reviewed the existing literature and

presented further observations. It is, therefore, considered unnecessary in this paper to review the literature. The results of other workers will be referred to when comparison with the data presented is considered appropriate.

The present study arose out of interest in a possible association between the pituitary gland and the ovary in dairy cattle affected by some form of ovarium dysfunction. To provide a background for such a study it was considered desirable to obtain detailed information on a large sample of cattle pituitaries. This paper summarizes weight and measurement data of nearly 1,300 pituitaries from cull dairy cows and beef steers. In addition, comparative data are presented for glands from a smaller number of animals for which the sexual state or experimental treatment was known. The experimental treatment involved the use of hormone preparations of various types. A detailed histological study on some of this material will be reported elsewhere (Bassett, 1951).

MATERIAL AND METHODS.

Some 2,000 cattle pituitaries were collected in the course of investigations on the production of pituitrin from New Zealand cattle which was undertaken as a war emergency project. These glands were derived from cull dairy cows and export-quality beef steers slaughtered at the Westfield Freezing Works, Auckland.

Of the 1,282 glands which were examined in detail, 902 were from dairy cows, mainly Jersey or Jersey Grade, with not more than ten per cent Friesian or Ayrshire. These animals were all mature (6-10 years of age) and about thirty per cent were from 6 to 7 months pregnant. Glands from 380 beef steers, mostly of the Hereford breed, were also examined. These were mainly 4- to 5-year-olds. A few glands from beef cows were also obtained, though as this sample was a small one, the figures presented must be accepted with reservation.

Owing to practical difficulties resulting from the "chain" system of slaughter employed, it was impossible to relate glands to the individual animals from which they were derived. It was possible only to ensure that glands from dairy cows were distinguished from beef-type animals. The only valid comparison that can be made is between these two types.

Each gland was recovered within half an hour of slaughter by the use of a special machine designed to shear the skull longitudinally without cutting the gland. The glands were removed from the cracked skull with a knife, trimmed immediately of the bulk of associated capsular and vascular tissue, weighed, graded for shape, measured for maximum length, width and depth, and fixed in acetone for subsequent preparation of pituitrin. On return to the laboratory (approximately eight hours later), the posterior lobes of the now partially dehydrated glands were dissected out, and the weights of anterior and posterior lobes were recorded. During collection, approximately every tenth gland was retained for more detailed examination, being placed in fixative immediately after measurements had been completed.

Subsequently, in the course of collection for histological study of glands from cattle in various reproductive conditions, whole trimmed pituitary weights were recorded for 200 dairy cows, 13 heifer calves and 13 bulls (Table VII.) The exact ages were not known in all cases, but detailed information was available as to the condition of the reproductive organs and/or the previous experimental treatment. The weights have been grouped accordingly and the means compared. In all cases standard errors of individual observations have been calculated.

All these cows were in final stages of, or had just completed, a lactation. The first four and the last five groups require no further dsecription than that given in Table VII. The animals in Group 5 were each injected with a total of 1,120mg of stilbestrol every second day over a period of twelve weeks prior to slaughter. Before treatment all ovaries of these animals were considered to be normal and functional as determined by rectal palpation. Without exception, the ovaries at slaughter were very small and quiescent, with only a few very immature follicles and no corpora lutea present. Cows in Group 6 were injected with 360mg of stilbestrol over 15 weeks. Of these, three developed cystic follicles but the ovaries of the remainder were apparently normal.* Group 7 consisted of cows each injected once with pregnant-mare serum (1,500 units) and killed 28 days later. Five of these showed superovulation, two had cystic ovaries, and the ovaries of the remainder appeared to be normal. The cows in Group 8 had been fed thiourea for periods ranging from 8 to 56 days with dosage range from 105 to 560 g. Those in Group 9 were fed thryoprotein for periods ranging from 14 to 56 days with doseage range of from 140 to 840 g.*

RESULTS.

Glands Collected from Westfield Freezing Works.

The Shape of the Pituitary.

Glands were classified as "round", "oval", or "pear", according to shape. Naturally some degree of variation and overlap existed within and between shapes. Considerable variation occurred both within and between the two main groups of cattle. Glands from dairy cows tended to be oval or round in shape, while those from beef steers were mostly pear-shaped. Gilmore *et al.* (1941), working on a small number of beef- and dairy-cow glands likewise reported a considerable shape variation, but gave no comparative description. Table 1 shows the shape distribution of the glands of this study.

TABLE 1. SHAPE OF PITUITARY GLANDS.

Class	Glands	Round (%)	Oval (%)	Pear (%)
Dairy Cows	902	16.8	46.8	36.4
Beef Steers	260	5.4	24.6	70.0
Beef Cows	12	16.7	66.8	16.7

*Material in Groups 6 and 8 supplied by I. L. Campbell and M. G. Holland, Dairy Res. Institute, Palmerston North.

TABLE II. LINEAR DIMENSIONS OF PITUITARY GLANDS

Class	Gland Shape	No. of Glands	Length (mm)	Width (mm)	Depth (mm)
Dairy Cows	Pear	326	23.0 ± 1.9	15.2 ± 1.8	11.9 ± 1.6
	Oval	428	22.5 ± 1.2	16.0 ± 1.6	12.7 ± 1.7
	Round	148	22.0 ± 1.9	18.0 ± 1.7	13.3 ± 1.6
Mean dimensions		902	22.5 ± 1.5	16.0 ± 1.8	12.5 ± 1.8
Beef Cows	Chiefly Oval	12	22.2 + 2.0	16.7 + 1.8	12.6 + 1.3
Beef Steers (4-5 years)	Pear	182	22.7 + 1.8	14.7 + 1.8	12.2 + 1.2
	Oval	64	22.0 + 1.8	15.8 + 1.6	12.3 + 1.3
	Round	14	20.4 + 1.2	17.4 + 1.5	12.9 + 1.1
Mean dimensions		260	22.4 + 1.8	15.1 + 1.8	12.3 + 1.3
		COMPARABLE DATA FROM OTHER SOURCES			
* Jerseys		84	22.1	17.0	14.6
+ Mainly Guernseys		12	20.7 ± 2.7	16.4 ± 2.1	13.5 ± 1.4
+ Beef Cattle (mixed breeds)		13	19.7 ± 1.2	15.1 ± 0.8	12.5 ± 1.2

* Swett *et al.* (1937)

\+ Gilmore *et al.* (1941)

Compared with dairy-cow glands, beef-steer glands showed approximately twice the proportion of pear-shaped, half the proportion of oval-shaped, and one third the proportion of round-shaped glands. The beef-cow glands were mainly oval-shaped.

From observations during dissection, it appeared that differences in shape were due to variation in the relative development of the anterior and posterior lobes. This is confirmed by the relative weights of these lobes (Table V). The smaller anterior lobe and the larger posterior lobe of the steer pituitaries resulted in the posterior lobe protruding further from the main body of the gland, thus giving the characteristic pear-shape. Conversely, the oval or round shape of dairy-cow glands arose from a greater relative development of the anterior lobe, which accordingly surrounds the posterior lobe more completely. Thus gland shape depends upon relative development of both posterior and anterior lobes.

The Dimensions of the Pituitary.

The linear measurements of the same glands are shown in Table II, the maximum dimensions in all cases being given. It will be noted that these measurements provide a factual basis for the visual shape gradings.

There appear to be no differences between glands of dairy and beef cows, but dairy-cow glands tend to be larger in all measurements than the beef-steer glands, though this difference is marked only in respect to width. The difference of 1 mm is highly significant statistically. Within these groups the pear-shaped glands are longest, the round-shaped glands shortest, widest and deepest. These differences are also highly significant, except in the case of the steer glands, where the significance of the difference between the width of pear- and round-shaped glands falls to the five per cent level.

Little information has been recorded previously on pituitary measurements. Swett *et al.* (1936) gave measurements for several dairy-cow breeds, though they included no figures for beef cows, or for males. Gilmore *et al.* (1941) published measurements of twelve dairy-cow and thirteen beef-cow glands.

From their raw data it would appear that the glands from beef cows are slightly smaller in all dimensions than those from dairy cows, though the differences are not significant.

The data of these workers are included in Table II.

The Weight of the Pituitary.

All glands were weighed in the fresh trimmed condition. Since these weights included part of the capsule, which could not be completely removed without injury to the gland, the weights for true gland tissues were calculated from the relative gland and capsule weights of one hundred glands of which the fresh weights were known and which were partially dehydrated in acetone for approximately six hours. Fresh weights were then corrected on the assumption that both capsular and gland tissue were dehydrated to the same extent. Thus, weights recorded for true gland tissue may be subject to a small error. Table III, gives the weights of trimmed fresh glands, and calculated weights of true fresh gland tissue. Table IV summarizes comparable data from other sources

TABLE III. WEIGHTS OF PITUITARY GLANDS (WESTFIELD)

Class	Gland Shape	No. of Glands	Trimmed Gland Weight (Fresh) (g)	Estimated True Gland Tissue Weight (Fresh) (g)	Av. Dressed Weight of Cattle (lb)
Dairy Cows	Pear	326	2.6 ± 0.54		
	Oval	428	2.8 ± 0.62		
	Round	148	3.2 ± 0.60		
Mean dimensions		902	2.80 ± 0.61	2.37	386
Beef Cows	Chiefly Oval	12	2.76 ± 1.97	2.37	600
Beef Steers	Pear	182	2.4 ± 0.13		
	Oval	64	2.6 ± 0.40		
	Round	14	2.7 ± 0.34		
Mean dimensions		260	2.51 ± 0.32	2.07	708

TABLE IV. WEIGHTS OF PITUITARY GLANDS OF ANIMALS COMPARABLE IN RESPECT TO AGE AND BREED

Source of Data	Class	Breed	Sexual Condition	No. of Glands	Pituitary Wgt. (g)
Wittek (1913)	Dairy Cows	Not recorded	Multiparous, non-pregnant.	148	2.79
	Bulls (4-5 Yrs)	"		101	3.0
Swett et al. (1937)	Dairy Cows	Jersey	Parous, non-pregnant.	210	2.8
*Reece & Turner (1937)	Dairy Cows	Mixed	Some lactating but non-pregnant. Some lactating and pregnant. Some dry and non-pregnant. Some dry and pregnant.	50	1.75
	Beef Cows	Mixed	" "	22	1.87
	"	Hereford	" "	13	1.87
	Beef Steers	Mixed		42	1.21
	"	Hereford		16	1.18
*Lewis & Turner (1939)	Dairy Cows	Mixed	Parous, non-pregnant; some lactating, some dry.	17	2.33± 0.77
	"	Mixed	Pregnant.	16	2.35± 0.52
	Beef Cows	Mixed	Pregnant.	22	2.20± 0.47
	"	Mixed	Non-pregnant.	18	2.04± 0.45
	Beef Steers (Over 700 lb)	Mixed		37	1.1 ± 0.25
Gilmore et al. (1941)	Dairy Cows	Jersey	Not pregnant	14	2.57
	Beef Cows	Mostly Shorthorn and Hereford.	Heifers	13	1.92
Bassett et al.	Dairy Cows	Mainly Jersey	Many pregnant - all at end of or just finished a lactation	902	(2.80± 0.61 (2.37*
	Beef Cows	Hereford	Dry	12	2.76± 1.97
	Beef Steers	Mainly Hereford		260	(2.51± 0.32 (2.07*

* Membranes removed.

The mean differences in weights of glands of different shapes show that the round glands tend to be heaviest and the pear-shaped glands lightest. The dairy-cow glands are significantly heavier than the beef-steer glands; when gland weight is expressed as a percentage of the dressed-carcass weight this difference is even more marked. The figures given by Reece and Turner (1937), and Lewis and Turner (1939) for similar types of animals also differ in this way. The beef cows yielded glands of approximately the same weight as those from the dairy-cow group. Though small mean differences between beef- and dairy-cattle glands are recorded by Reece and Turner (1937), and Lewis and Turner (1939), our statistical analysis of their figures showed these to be not significant. Mean figures for these two types taken from raw data of Gilmore *et al.* (1941) show a weight difference in favour of dairy cows, but the two groups are not comparable in respect to age.

The Weight of Anterior and Posterior Lobes.

Table V gives anterior and posterior lobe weights based on partially dehydrated weights and thus subject to errors due to possible differences in extent of dehydration. Similar treatment in respect to storage-time, dissection and weighing were employed to reduce errors to a minimum, and the large numbers upon which the data are based are likely to smooth out most of the variation due to these causes.

TABLE V. RELATIVE WEIGHTS OF ANTERIOR AND POSTERIOR LOBES OF PITUITARY GLANDS

	DAIRY COWS	BEEF STEERS
Number of animals	880	380
Weight of dehydrated lobes		
Anterior lobe	1.20 g	0.97 g
Posterior lobe	0.18 g	0.21 g
Estimated weight of fresh lobes		
Anterior lobe	2.06 g	1.71 g
Posterior lobe	0.31 g	0.36 g
Percentage of total weight of lobes		
Anterior lobe	86.9	82.5
Posterior lobe	13.1	17.5
Relative weights of lobes		
Anterior lobe	100	81
Posterior lobe	100	117

TABLE VI. COMPARATIVE WEIGHTS OF ANTERIOR AND POSTERIOR LOBES.

Source of Data	* Class	No. of Glands	Average weight of glands			Percentage of total weight		Relative weight of lobes	
			Ant.lobe (g)	Post.lobe (g)	Total (g)	Ant. lobe	Post. Lobe	Ant. lobe	Post. lobe
** Reece & Turner (1937) Hereford	Dairy cows	50	1.46	0.29	1.75	83.4	16.6	100	100
	Beef cows	13	1.59	0.28	1.87	85.0	15.0	109	96
	Beef steers	16	0.96	0.22	1.18	81.4	18.6	65	75
** Lewis & Turner (1939) Mixed	Dairy cows	17	1.89	0.44	2.33	81.1	18.9	100	100
	Beef cows	18	1.73	0.31	2.04	84.8	15.2	91	70
	Beef steers	37	1.01	0.08	1.09	92.7	7.3	53	18
Gilmore et al. (1941)	Dairy cows	11	1.86	0.20	2.06	90.3	9.7	100	100
	Beef cows	7	0.86	0.35	1.21	71.1	28.9	46	175
** New Zealand *** (Westfield data)	Dairy cows	880	2.06	0.31	2.37	86.9	13.1	100	100
	Beef steers	380	1.71	0.36	2.07	82.5	17.5	81	117

* For details of sexual condition see Table IV.

** Membranes removed.

*** Estimated weight. See text.

These figures show that, as was indicated by the shape gradings and the measurement data, the greater whole weights of dairy-cow glands as compared with those from beef steers may be ascribed to the relatively greater anterior-lobe weight in the former. Differences in weight between differently shaped glands are due to the same cause. Rather than being indicative of a sex or castration effect, this difference is more probably due to the influence of lactation in the female. Lewis and Turner (1939), who record a great increase in absolute and proportional pituitary weight in parous over nulliparous cattle, consider that this increase must occur during lactation, and that while there is only a temporary hypertrophy of the anterior lobe the posterior lobe is permanently enlarged.

The posterior lobes of cow pituitaries are lighter than those of steers. Lack of similar data for bulls or nulliparous cows prevent any satisfactory interpretation of these figures in terms of physiological differences.

The data of other workers shown in Table IV present a confusing picture when considered in relation to these results, though their observations are in all cases from a smaller number of animals.

TABLE VII. WEIGHTS OF PITUITARIES IN SELECTED ANIMALS

	Animal Group	No. of Animals	Mean Pituitary Weight	Significance	
1.	Cows with normal ovaries (dry)	85	2.93 ± 0.66		
2.	Cows pregnant	31	3.05 ± 0.62	N.S.	
3.	Cows with cystic follicles	16	3.85 ± 0.54	S.S.	
4.	Cows with cystic Corpora lutea	12	3.15 ± 0.44	N.S.	
5.	Cows injected with stilbestrol at high dose rate	9	3.66 ± 0.75	S.	
6.	Cows injected with stilbestrol at low dose rate	9	3.81 ± 0.55	S.S.	
7.	Cows injected with pregnant mare serum	23	2.77 ± 0.79	N.S.	
8.	Cows fed with thiourea	7	2.79 ± 0.57	N.S.	
9.	Cows fed with Thyroprotein	8	2.81 ± 0.73	N.S.	
10.	Female calves under 3 weeks	13	1.42 ± 0.22	S.S.	
11.	Bulls sterile	5	5.19 ± 0.71	S.S.	*
12.	Bulls fertile	7	2.52 ± 0.35		
13.	Bulls (Groups 11 and 12 combined)	12	3.63 ± 0.51	S.S.	**
14.	Cows (Normal, non-pregnant, same ages as in Group 13)	12	2.98 ± 0.72		

* Compared with fertile bulls.
** Compared with cows of same ages.

Results from Examination of Selected Animals.

Table VII shows mean weights and associated standard errors of pituitary weights from animals grouped according to reproductive condition or experimental procedure. There is no weight-difference between glands from pregnant cows and those from

normal non-pregnant animals, a result in agreement with that of other workers. (Wittek, 1913; Reece and Turner, 1937; Lewis and Turner, 1939.) Weights of glands from cows with cystic follicles and from cows injected with stilbestrol at both high and low rates are significantly greater than those from normal non-pregnant animals, while for cows with cystic corpora lutea there is no such difference. No previous reference can be found concerning the effect on the cattle pituitary of administered oestrogens, nor do there appear to have been any observations made on pituitaries from females of any species with cystic ovaries. Blickenstaff (1934) reported that pituitaries from sterile cows, whose ovaries showed that they had not ovulated for some time, were heavier than those from cows with normally functioning ovaries. Increase in pituitary-weight following treatment with oestrogenic hormones has been reported for other species by many workers. (For rats, Cramer and Horning, 1936; McEwen *et al.*, 1936; Halpern and D'Amour, 1936; Emery, 1943; and others.) There is no significant difference between any of the other experimental groups and the normal.

Though sterile bulls appear to have significantly heavier pituitaries than normal bulls, the true position is confused by the fact that the sterile group consisted of older animals than those in the fertile group, which were all under four years of age. The largest pituitary-weight to be recorded during the investigation, but not included in the above table, was that of a pedigree Jersey Bull of eleven years, sterile during its last year of life. The gland weighed 8.6 g. An approximate comparison between the pituitary weights for bulls and cows has been made by combining the two bull groups and comparing the mean with that derived from a group of cows of equivalent ages. Though bull glands are slightly heavier in absolute weight, when the average body-weights of the two sexes, viz. 900 lb for cows and 1,400 lb for bulls, are taken into consideration it is found that the proportional weight of the glands of bulls is less than that of cows. Lewis and Turner (1939) found that in bulls proportional pituitary weight was considerably lower than in pregnant and non-pregnant dairy cows, but approximately equal to that in dry dairy cows in the follicular stage of the oestrous cycle. The average age of the fertile bull group is similar to that of the steer group in Table IV, and there is no significant difference in absolute pituitary weight. However, when considered in relation to body weight the proportional pituitary weight is greater for steers, though the difference in size of the two samples is too great for a valid comparison to be made. Though Wittek (1913) found that pituitary weight per unit weight of dressed carcass was greater for steers than for bulls, Reece and Turner (1937), and Lewis and

Turner (1939) found little difference in pituitary weight between mature steers and bulls.

Examination of Gross Histological Features.

Sagittal sections taken through a large number of whole glands were made in order to observe the macroscopic appearance and the relative proportions of the three main components. As was indicated by the weight data (Table V) in both dairy cows and beef steers, the anterior lobe accounts for the greater part of the gland, as has been described by other workers, including Herring (1908), Trautman (1909), Rasmussen (1934), and Lewis and Turner (1939).

In this investigation the anterior lobe and the *pars intermedia* were found to be better developed in the dairy cows than in the steers. In addition there was greater vascularization of the pituitaries from the dairy cows. The cone of Wulzen (Wulzen, 1913) was observed in nearly every gland examined, while the cleft between the two lobes was well defined in each case.

Figure 1 illustrates sections through both normal and abnormally large glands. In dairy-cow glands this increase in size was often found to be related to the presence of a "colloid" body, or cyst, in the cleft between the two lobes. In a count of 300 glands of each type, 33 per cent of dairy-cow glands contained these bodies, as against 2 per cent of the glands from beef steers. These masses were much larger in pituitaries from cows, being as large as a garden pea in many cases. In the case of glands classified as "abnormally large", these cysts could be seen by external examination. At first it was considered that the high percentage of colloid cysts in glands from New Zealand dairy cows might have some relation to the high incidence of sterility, but subsequent examination of glands from animals whose physiological condition was known, showed that the cysts were apparently not related to infertility, as they occurred in glands from pregnant cows as well as in glands from sterile animals.

Cysts in the cleft between the anterior and posterior lobes of the pituitary have been reported for cattle by several workers, including Sutton *et al.* (1940), Marsden *et al.* (1942), and Hodson *et al.* (1946). In these reports such cysts have been related to Vitamin A deficiency in the animal and occurred in glands from animals of both sexes. Pressure atrophy of the surrounding tissue was observed in many cases and associated cellular changes occurred. Only five cysts were reported in glands from 10,000 apparently normal cattle (Marsden *et al.*, 1942). Detailed histological examination of cystic glands examined in this investigation showed no pressure atrophy or cellular changes in the glandular tissue (Basset,

1951). For these reasons and since there is little likelihood of any Vitamin A deficiency in New Zealand grass-fed cattle, it is considered that the cysts recorded here are not the same as those previously reported in the literature.

REFERENCES.

(1) BASSETT, E. G. (1951): J. Endocrinology. (In Press.)
(2) BENNETS, H. W.; UNDERWOOD, E. J.; and SHIER, F. L. (1936): Aust. Vet. J., 22, 2.
(3) BLICKENSTAFF, P. H. (1934): Vet. Alumni Quarterly, 21, 132.
(4) CRAMER, W.; and HORNING, E. S. (1936): Lancet, 1, 1056.
(5) FICHER, G. (1905): Cited by Gilmore et al. (Reference No. 6.)
(6) GILMORE, L. O.; PETERSEN, W. E.; and RASMUSSEN, A. T. (1941): Univ. Minn. Tech. Bull. 145.
(7) HALPERN, R. S.; and D'AMOUR, F. E. (1936): Am. J. Physiol, 115, 229-38.
(8) HERRING, P. T. (1908): Cited by Gilmore et al. (Ref. No. 6.)
(9) HODGSON, R. E.; HALL, S. R.; SWEETMAN, W. J.; WISEMAN, H. G.; and CONVERSE, H. (1946): J. Dairy Sci., 29, 669.
(10) KOHN, A. (1910): Cited by Gilmore et al. (Ref. No. 6.)
(11) LEWIS, A. A.; and TURNER, C. W. (1939): Univ. Mo. Res. Bull. 310.
(12) MARSDEN, L. L.; Hall, S. R.; and CONVERSE, H. T. (1942): J. Nutrition, 24, 15.
(13) McEUEN, C. S.; SELYE, H.; and COLLIP, J. B. (1936): Lancet, 1. 755.
(14) RASMUSSEN, A. T. (1934): Am. J. Anat., 55, 253-75.
(15) ———————— (1938): Proc. Assoc. for Res. in Nervous and Mental Disease, 17, 118-50.
(16) REECE, R. P.; and TURNER, C. W. (1937): Univ. Mo. Res. Bull. 226.
(17) ROSTA, M., (1931): Cited by Gilmore et al. (Ref. No. 6.)
(18) SUTTON, T. S.; KRAUSS, W. E.; and HANSARD, S. L. (1940): J. Dairy Sci., 23, 574.
(19) SWETT, M. W.; MATTHEWS, C. A.; MILLER, F. W.; and GRAVES, R. R. (1937): Bur. Dairy Industry M-589. (Revised.)
(20) TRAUTMAN, A. (1909): Cited by Gilmore et al. (Ref. No. 6.)
(21) WITTEK (1913): Cited by Reece and Turner, (Ref. No. 16), and by Gilmore et al., (Ref. No. 6).
(22) WULZEN, R. (1914): Anal. Rec., 8, 403.

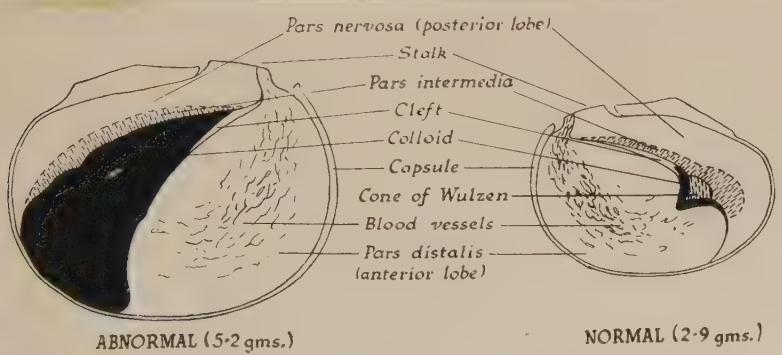

Fig 1. SAGGITAL SECTIONS OF PITUITARY GLANDS OF COWS.

RIBOFLAVIN CONTENT OF NEW ZEALAND MILKS.

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Summary.

Bulked milk obtained from the largest milk distributors in the four main centres in New Zealand has been tested microbiologically for its riboflavin content before and after pasteurization. An average figure for the riboflavin content of New Zealand milk may be taken as 2.0 μg per gram, while a pint may be considered to supply 1.17 μg on the average.

A variable, usually slight diminution in the riboflavin content following pasteurization, has been noted, but this may possibly be attributable to factors incidental to pasteurization rather than to the pasteurization process itself.

INTRODUCTION.

Milk is the food which should contribute the largest proportion of the riboflavin intake for the New Zealand population because, if one pint of milk is consumed daily, it is capable of supplying approximately one half of the daily requirement of the adult. McDowall, Bathurst, and Campbell (1947) have reported the riboflavin content of milk from Jersey and Friesian cows in New Zealand; Jersey milk varied from 0.19 to 0.33 mg per 100 ml, with an average of 0.255 mg per 100 ml, Friesian milk, from 0.14 mg to 0.28 mg with an average of 0.194 mg per 100 ml. As the proportion of these two and other breeds varies in the herds supplying town milk in the North and South Island, a representative figure for bulked milk was considered desirable. Samples were obtained from the largest milk distributors in the main centres. These distributors have pasteurizing plants and so the opportunity was taken to compare the riboflavin values of the milk before and after pasteurization.

EXPERIMENTAL METHOD.

Selection of Samples.

The samples consisted of raw bulk-milk and the same bulk-milk after pasteurization. These were collected in dark bottles and kept in a refrigerator prior to forwarding.

Collection of the Dunedin milk was made personally early in the afternoon in order to secure milk that had been brought in and pasteurized the same morning. The milk from Auckland, Wellington, and Christchurch, was received by air-mail in the afternoon on the day of despatch. Wellington and Christchurch milk had been pasteurized that morning, but Auckland milk was a day old on arrival.

Preparation of Sample for Test.

Five millilitres of whole milk were diluted with 60 ml distilled water, the whole autoclaved at a pressure of 15 lb per sq. in. for 15 min, cooled, adjusted to pH 6.6-6.8 using brom-thymol blue as an external indicator, and made up to 100 ml in volume. Aliquots of 1 ml, 2 ml, and 3 ml, each in triplicate, were added to the assay tubes.

Comparison of results following this procedure was made with results of other methods. The first of these methods was that followed by Snell and Strong (1939). It was the same as the one described except that autoclaving was omitted. In the second method, 5 ml milk were autoclaved with 60 ml 0.1 N H_2 SO_4, cooled, adjusted to pH 6.6-6.8 as before and made up to 100 ml in volume. This was the method used by Keely (1944). In the third, the fat was removed from the milk by centrifuging, before autoclaving with acid as above (Barton-Wright and Booth, 1943).

Method of Test.

The microbiological method of Snell and Strong (1939) was followed. Since Bacto yeast was not available, D.Y.C. yeast, a New Zealand commercial dried yeast, was used instead. Determinations of the water-soluble matter were first necessary, and calculations of the weight required in the preparation of the yeast supplement were then made. Because of the long, laborious procedure involved in its preparation, the technique of Strong and Carpenter (1942) was adopted, i.e. the first filtering after the addition of basic lead acetate was omitted.

The riboflavin standard, if kept in a dark bottle in a refrigerator, was found to remain unchanged after ten weeks.

As a nephelometer was not readily available only acid production was measured. Triplicates for each level of concentration as used by Barton-Wright and Booth (1943) were deemed more satisfactory than the duplicate levels used in the original method.

The organism used was *Lactobacillus casei* ℰ obtained from the Dairy Research Institute (N.Z.), Palmerston North. Barton-Wright and Booth's method of preparing inocula was followed to minimize the risk of contamination.

Recovery experiments were done in a number of instances to check the results of the milk assays.

RESULTS.

Unsatisfactory results were obtained when the milk was diluted with distilled water and not autoclaved, and when the fat was removed. The other procedures, viz. autoclaving with distilled water and autoclaving with acid, gave consistent and satisfactory results.

The results are recorded in Table I.

TABLE I. COMPARISON OF THE RIBOFLAVIN CONTENT OF RAW AND PASTEURIZED MILK

Source	Raw Milk (μg / ml)	Pasteurized milk (μg / ml)	Loss due to Pasteurization (%)
DUNEDIN			
Mean	1.98	1.79	9.5
Range	1.87 - 2.20	1.69 - 2.01	4.7 - 13.6
Standard Deviation	0.081	0.080	
WELLINGTON			
Mean	2.20	2.09	4.9
Range	2.15 - 2.25	2.04 - 2.12	2.3 - 8.9
Standard Deviation	0.038	0.026	
CHRISTCHURCH			
Mean	2.14	2.01	6.3
Range	2.08 - 2.18	1.97 - 2.05	5.3 - 7.8
Standard Deviation	0.038	0.033	
AUCKLAND			
Mean	2.14	2.01	6.8
Range	2.05 - 2.20	1.96 - 2.06	3.8 - 14.5
Standard Deviation	0.054	0.040	

Note:- The percentage recovery in each case ranged from 94 to 107 per cent with an average of 101 per cent.

DISCUSSION.

The results of workers in U.S.A. vary from 1.5 to 2.5 μg per gram. The range of figures in the study reported here is from 1.87 to 2.25 μg per millilitre for raw milks, and 1.69-2.12 for pasteurized milks. When the figures obtained for Jersey and Friesian milks by McDowall, Bathurst and Campbell (1947) are calculated per gram of milk on the basis of a specific gravity of 1.033, they range from 1.84 to 3.19 μg per gram of Jersey milk with an average of 2.47; and from 1.35 to 2.71 for Friesian milk with an average of 1.88 μg per gram. A general average figure for New Zealand milk might

be taken as 2.0 μg riboflavin per gram, while a pint might be taken to contain 1.17 μg, i.e. over 50 per cent of the adult daily requirement of riboflavin.

Concerning the effects of pasteurization, opinions differ as to whether there is any diminution in the riboflavin content of milk as a result of pasteurization. Zeigler and Keevil (1944) state that 9-16 per cent is lost; but in carefully controlled experiments where milk was pasteurized by the spray vat method, the coil vat method, and the flash process, Holmes (1944) has shown that negligible loss occurs with pasteurization. In the study here reported, the samples were taken from bulk milk before and after pasteurization, and no information is available as to the amount of light that might have reached the milk in the time between taking the two samples. It is, therefore, impossible to say whether the decrease in riboflavin content observed in the pasteurized milk was due to the pasteurization process or to other factors such as destruction by light at any stage of the process.

REFERENCES.

(1) BARTON-WRIGHT, E; and BOOTH, R. G. (1943): Biochem. J., 37, 25.
(2) HOLMES, A. D. (1944): J. Am. Dietetic Assoc., 20, 226.
(3) KEELY, Margaret (1944): Biochem. J., 38, 423.
(4) McDOWALL, F. H.; BATHURST, N. O.; and CAMPBELL, I. L. (1947): N.Z. J. Sci. and Tech., 28 (Sec. A), 316.
(5) SNELL, E. E.; and STRONG, F. M. (1939): Ind. Eng. Chem. (Anal. Ed.), 11, 346.
(6) STRONG, F. M.; and CARPENTER, L (1942): Ibid, 14, 909.
(7) ZIEGLER, J. A.; and KEEVIL, W. B. (1944): J. Biol. Chem., 155, 605.

STUDIES ON SOME NEW ZEALAND ROMNEY MARSH STUD FLOCKS.

PART IV. LAMBING SEASON.

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Summary.

(1) The average length of gestation, based on 261 ewes of all ages, was found to be 148.2 ± .14 days. The effects of season, type of birth and age of dams on the length of gestation have been analysed.

(2) The lambing season based on 2,236 ewes lasted 13 weeks, beginning usually in the second week, and reaching its peak in the last week, of August.

(3) The influence of age of dams on date of lambing was found to be statistically significant for the first four weeks of the lambing season. There was also a tendency for twin births to occur more often than single births, during this period.

INTRODUCTION.

The present paper concludes the analysis of the reproductive cycle of some New Zealand Romney Marsh Stud sheep. It is divided, for the sake of convenience, into two sections: A. Gestation, B. Lambing season.

The tupping season has been described previously (Goot, 1949), and its relationship with the lambing season will be discussed elsewhere.

In accordance with common usage the age terminology adhered to refers to the approximate age of ewes at mating; five months must obviously be added to arrive at the age of ewes at lambing.

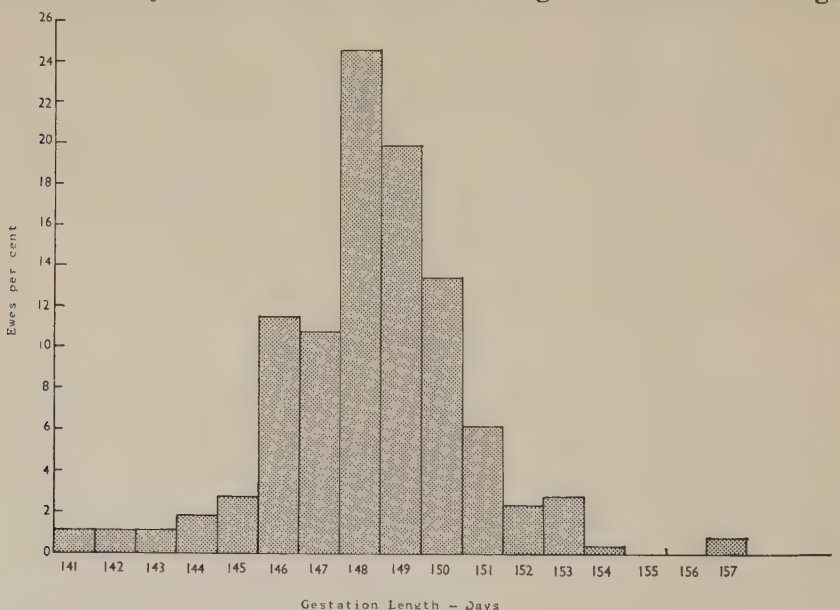

FIG. 1. LENGTH OF GESTATION PERIOD IN EWES.

GESTATION.

The length of gestation, i.e., the time interval between the last tupping and lambing, has been obtained for 261 ewes, which were hand-served in Stud B (Goot, 1946) in the 1937 and 1945 seasons.

The results are summarized in Table I and shown graphically in Fig. 1. The length of gestation periods ranged from 141 to 157 days, with a mean of 148.21 ± .14 days. This is substantially the same figure as obtained in this country by Gill (1933), Dry (1933), and Webster (1937) for Romney sheep, *viz.* 148.75, 148.7 and 148.88 days respectively.

The results of other investigations have been compiled by Kenneth (1943), and Terrill and Hazel (1947).

The following factors which may influence the gestation period were statistically analysed, and differences between means tested for significance using Student's t-test (Snedecor, 1940).

Season.

The difference between the 1937 and 1945 seasons was found to be small and statistically non-significant (Table I).

TABLE I. LENGTH OF GESTATION PERIODS IN EWES ACCORDING TO SEASON, TYPE OF BIRTH, AND AGE.

Season, type of birth and age		No. of gestations	Mean days	S.D. days	S.E. days
SEASONS:	1937	152	148.05	2.49	0.20
	1945	109	148.57	2.05	0.20
TYPE OF BIRTHS:					
Single births:	Male	61	148.74	2.01	0.26
	Female	79	148.33	2.47	0.28
All single births		140	148.51	2.27	0.19
Twin births:	Male Twins.	27	147.78	2.10	0.40
	Female Twins	28	148.57	2.96	0.56
	Mixed Twins	55	147.75	2.19	0.30
All twin births		110	147.96	2.39	0.23
	Triplets	11	147.29	...	...
AGE:	2-tooth	96	147.65	2.31	0.24
	4-tooth	46	148.48	2.22	0.33
	6-tooth	48	148.29	2.11	0.31
	Full-mouth and 5½ yr	37	148.46	2.02	0.33
	6½ yr and over	34	149.03	1.95	0.33
All births		261	148.21	2.21	0.14

Type of Birth.

In all, 28 comparisons were made, the number of triplet births being too few to be included. Only the significant results are summarized in Table II. Ewes giving birth to male singles had gestation periods significantly longer than ewes giving birth to male twins, to mixed twins, and to all twins. Further, ewes bearing male or female singles had gestation periods significantly longer than ewes bearing mixed twins.

FIG. 2. INFLUENCE OF AGE OF EWES ON TIME OF LAMBING AND TYPE OF BIRTH.

The significant differences between the length of gestation in ewes bearing male singles and twins, or singles and mixed twins, seem to be at least in partial agreement with findings of Vasin (1940) but at variance with those of Bonsma (1939) and Kelley (1943). On the other hand, the difference in the length of gestation periods between male and female births was non-significant, this being in accord with results of Bonsma (1939), Kelley (1943), and Terrill and Hazel (1947), but not with those of Vasin (1940).

Age of Dams.

Of the 12 comparisons tested, in only 4 cases were the differences statistically significant; these are shown in Table II. Apparently the gestation period in maiden ewes is somewhat shorter (Table I) than in older ewes. This conclusion is at variance with that of Bonsma (1939) who found no statistically significant differences between the first and subsequent pregnancies. Vasin (1940), and Terrill and Hazel (1947), however, concluded that the length of gestation increases with the age of dams.

Because of the contradictory results thus far obtained, and also because of small numbers available for some of the statistical comparisons, the present findings must be regarded as preliminary.

TABLE II. DIFFERENCES BETWEEN MEANS IN THE LENGTH OF GESTATION PERIODS. NOT SIGNIFICANT RESULTS ARE EXCLUDED FROM THIS TABLE.

			D.F.	t	Difference days
TYPE OF BIRTHS:					
Male single	vs.	Male twins	86	2.04 *	+ 0.96
Male single	vs.	Mixed twins	114	2.54 *	+ 0.99
Male single	vs.	All twins	169	2.16 *	+ 0.78
All singles	vs.	Mixed twins	193	2.13 *	+ 0.76
AGE OF DAMS:					
2-tooth	vs.	4-tooth	140	2.03 *	- 0.83
2-tooth	vs.	6½ yr and over	128	3.11 **	- 1.38
2-tooth	vs.	4-tooth to 5½ yr	225	2.54 *	- 0.75
2-tooth	vs.	4-tooth to 6½ yr and over	259	3.15 **	- 0.88

* Significant at 5 per cent level.
** Significant at 1 per cent level.

LAMBING SEASON.

The material consisted of 809 ewes which lambed in the 1937, 1944 and 1945 seasons in Stud B, and 1,427 ewes which lambed in 1935-41 and 1944-45 seasons in Stud C (Goot, 1946).

It will be remembered (Goot, 1949) that the tupping season was defined as that part of the sexual season during which the rams are run with the ewes; it is, therefore, to a certain extent arbitrary. Consequently, the beginning, duration and the end of the lambing season is dependent upon the tupping season. The general relationship between these two seasons will be discussed elsewhere (Goot, 1951).

The lambing season in both Studs lasted about 13 weeks, beginning usually in the second week of August and ending late in October or early in November. The number of ewes lambing in any one year was too small to enable seasonal comparisons to be made. The distribution of lambing, in weekly intervals, for both studs is given in Table III; the averages, based on total number

of ewes are also quoted. These averages, because of greater numbers in Stud C, are slightly biased towards the results of this stud. If the season is divided into three practically equal periods, it is found that over half of the ewes lambed in the first period, about one third to

TABLE III. DISTRIBUTION OF LAMBING IN WEEKLY PERIODS

Week No.	Date	Period	Stud B 809 ewes %	Stud B 809 ewes %	Stud C 1,427 ewes %	Stud C 1,427 ewes %	Both Studs 2,236 ewes %	Both Studs 2,236 ewes %
1	5.8 - 11.8	I	2.1	68.8	2.2	51.8	2.2	58.0
2	12.8 - 18.8		9.6		9.4		9.5	
3	19.8 - 25.8		27.1		17.4		20.9	
4	26.8 - 1.9		30.0		22.8		25.4	
5	2.9 - 8.9	II	9.0	23.5	13.2	39.0	11.7	33.5
6	9.9 - 15.9		5.2		11.0		8.9	
7	16.9 - 22.9		5.2		7.5		6.7	
8	23.9 - 29.9		4.1		7.3		6.2	
9	30.9 - 6.10	III	1.7	7.7	4.8	9.2	3.7	8.5
10	7.10- 13.10		2.4		2.0		2.1	
11	14.10- 20.10		2.1		1.9		2.0	
12	21.10- 27.10		0.6				0.4	
13	28.10- 3.11		0.9		0.5		0.3	

one fourth in the second, and less than one tenth in the third. It is of interest to note that the lower percentage of ewes lambing in Stud C during the first period was compensated for by an increase in the second period only, and in spite of the different distributions of lambing in Studs B and C, over 90 per cent of ewes lambed in each stud within the first two periods (eight weeks). The peak and the next-to-peak in both studs occurred in the fourth and third weeks respectively.

The percentage of ewes lambing singles and twins according to weeks and periods of the lambing season are given in Table IV. Owing to small numbers, ewes lambing triplets are not listed separately, but are included in the last column. The differences due to type of birth within the three periods were tested statistically using the chi-square method (Snedecor, 1940) and found to be non-significant except that the difference between the incidence of single and twin births occurring in the first period was close to the ten per cent point. It was reported by Heape (1899) that out of 275

TABLE IV. PERCENTAGE OF EWES LAMBED, IN STUD B, ACCORDING TO TYPE OF BIRTH

No. of ewes lambed and type of birth.		Singles				Twins										Total **		
		215 ♂		234 ♀		449 Total		86 ♂♂		166 ♂♀		90 ♀♀		342 Total				
Week No. *	Period	%		%		%		%		%		%		%		No.	%	
1	I	4.6	66.9	0.4	63.6	2.4	65.3	2.3	66.2	1.2	77.7	2.3	71.2	1.7	73.1	17	2.1	68.8
2		10.7		10.7		10.7		9.3		10.2		5.6		8.8		78	9.6	
3		26.5		25.6		26.1		26.7		26.5		32.2		28.1		219	27.1	
4		25.1		26.9		26.1		27.9		39.8		31.1		34.5		243	30.0	
5	II	6.5	24.2	12.4	27.0	9.6	25.7	11.6	29.1	7.8	17.4	5.6	21.1	8.2	21.4	73	9.0	23.5
6		6.5		4.7		5.6		9.3		1.2		7.8		5.0		42	5.2	
7		5.6		5.6		5.6		3.5		6.0		4.4		5.0		42	5.2	
8		5.6		4.3		4.9		4.7		2.4		3.3		3.2		33	4.1	
9	III	1.9	8.9	2.1	9.4	2.0	9.0	1.2	4.7	1.8	4.9	0.0	7.7	1.2	5.5	14	1.7	7.7
10		2.8		2.6		2.7		1.2		1.2		4.4		2.0		19	2.4	
11		2.8		1.7		2.2		2.3		0.7		3.3		1.7		17	2.1	
12		0.0		1.3		0.6		..		1.2		..		0.6		5	0.6	
13		1.4		1.7		1.5		..		..		..		..		7	0.9	
	TOTAL															809		

* For dates see Table III.
** Ewes lambing triplets included.

TABLE V. PERCENTAGE OF EWES, WITHIN EACH AGE-GROUP, LAMBING IN STUD B, ACCORDING TO TYPE OF BIRTH AND PERIOD OF THE LAMBING SEASON

Age and No. of ewes	2-tooth			4-tooth			6-tooth			Full Mouth & 5½ yr			6½ yr & over			Total		
	170	64	235*	127	64	192*	66	96	164*	53	82	145*	33	35	73*	449	342	809*
Type of birth	Single	Twins	All	Single	Twins	All	Single	Twins	All	Single	Twins	All	Single	Twins	All	Single	Twins	All
Lambing Season	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
Period I. 5.8 - 1.9	62	61	62	68	75	70	64	73	69	74	80	78	67	74	71	65.3	73.1	68.8
Period II. 2.9 - 29.9	26	31	27	27	20	25	27	21	24	22	16	18	18	12	15	25.7	21.4	23.5
Period III. 30.9 - 3.11	12	8	11	5	5	5	9	6	7	4	4	4	15	14	14	9.0	5.5	7.7

* Ewes lambing triplets included.

flockmasters who expressed their opinion on this point, more than half said that most twins are born early in the season. Similarly, Marshall (1908), Nikoljskii (1933), Arapov (1937) and Rehse (1938) have indicated that in general the proportion of twin births to single births is higher early in the season. On the other hand, Patov (1933) found no difference in the incidence of single and twin births throughout the lambing season.

A comparison of ewes, within each age group, lambing singles and twins is given in Table V. Here again, the tendency of twin births to occur early in the season is seen in all age groups, except in the two-tooth.

Table VI and Fig. 2 show the influence of the age of dams on the time of lambing. Only in the first period were the age-group deviations, irrespective of type of birth, from the mean of this period found to be statistically significant. This is undoubtedly due to the low value for two-tooth and high value for full-mouth and 5½-year-old ewes.

TABLE VI. PERCENTAGE OF EWES LAMBED IN STUD B, ACCORDING TO THEIR AGE, TYPE OF BIRTH, AND PERIOD OF THE LAMBING SEASON.

Age and No. of ewes	Type of Birth	2-tooth 235	4-tooth 191	6-tooth 162	Full Mouth & 5½ yr 135	6½ yr & Over 68	Total 791
Lambing season		%	%	%	%	%	%
Period I 5.8 - 1.9	Singles	45	45	26	29	32	37.3
	Twins	17	25	43	49	38	31.6
	Total	62	70	69	78	70	68.9
Period II 2.9 - 29.9	Singles	18	18	11	9	9	14.5
	Twins	9	7	12	10	6	8.9
	Total	27	25	23	19	15	23.4
Period III 30.9 – 3.11	Singles	9	3	4	1	7	4.9
	Twins	2	2	4	2	8	2.8
	Total	11	5	8	3	15	7.7
Total	Singles	72	66	41	39	48	56.7
	Twins	28	34	59	61	52	43.3

The frequency of the occurrance of single and twin births according to the age of dams is also shown in Table VI and Fig. 2. With advancing age of dams there is an increase in twin births and a decrease in single births within the first period. The reverse

trend seen in the second period is made up of a gradual decrease in the percentage of single births, the variations in twin births being probably of a random nature. Owing to the small number of ewes lambing in the third period, no interpretation is possible.

The age of dams seems also to affect the duration of lambing. Younger ewes, up to and including six-tooth, required 13 weeks to lamb; next came the age group of $6\frac{1}{2}$-year and older ewes with 12 weeks, while full-mouth and $5\frac{1}{2}$-year-old ewes lambed within 11 weeks. (Table VII.)

The results of further investigations will, it is hoped, be discussed in subsequent parts of this study.

TABLE VII. WEEKLY PER CENT OF EWES LAMBED IN STUD B ACCORDING TO AGE.

Age & No. of ewes.	2-tooth 235	4-tooth 192	6-tooth 164	Full Mouth & $5\frac{1}{2}$ yr 145	$6\frac{1}{2}$ yr & older 73	Total	
Week No.*	%	%	%	%	%	No.	%
1	1.7	2.6	1.8	1.4	4.1	17	2.1
2	9.7	8.2	9.7	14.4	4.1	78	9.6
3	31.1	24.7	26.7	24.7	30.1	219	27.1
4	19.8	34.5	30.9	37.7	32.9	243	30.0
5	5.5	12.9	9.1	10.3	6.9	73	9.0
6	7.2	6.7	5.5	2.0	1.4	42	5.2
7	6.0	3.6	6.1	4.1	5.5	42	5.2
8	8.4	1.6	3.6	1.4	1.4	33	4.1
9	2.1	2.1	0.6	2.0	1.4	14	1.7
10	4.2	0.5	2.4	..	6.8	19	2.4
11	1.3	1.6	1.2	2.0	2.7	17	2.1
12	0.5	..	1.2	..	2.7	5	0.6
13	2.5	1.0	1.2	..	..	7	0.9
Total						809	

* For dates see Table III.

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REFERENCES.

ARAPOV, P. V. (1937): Probl. Zitvotn. No. 3 64-85. (Abstract in Anim. Breed. Abst. 5, 283).

BONSMA, F. N. (1939): University of Pretoria Publications. Series 1: Agriculture No. 48, Pretoria, South Africa, pp. 214.

DRY, F. W. (1933): N.Z. J. Agr., 47, 386-7.

GILL, D. E. (1933): Ibid., 305-7.

GOOT, H. (1946): N.Z. J. Sci. & Tech., 28 (Sec. A), 83-93.
(1949): Ibid., 30 (Sec. A.), 330-44.
(1951): Ibid., (In the press).

HEAPE, W. (1899): J. Roy. Agr. Soc., X, 217-48.

KELLEY, R. B. (1943): Australian Vet. J., 19, 43-5.

KENNETH, J. H. (1943): "Gestation Periods, A Table and Bibliography." Oliver and Boyd Ltd., Edinburgh.

MARSHALL, F. H. A. (1908): Trans. Highland and Agr. Soc. Scot., 20. 139-51.

NIKOLJSKII, N. F. (1933): Trudy Inst. Gibrid. Askania Nova. 1, 251-67. (Abstract in Anim. Breed. Abst., 2, 121.)

PATOV, C. V. (1933): Z. Zuchtg., 26, 285-321. (Abstract in Anim. Breed. Abst., 1, 19.)

REHSE, H. (1938): Z. Schafz., 27, 218-23. (Abstract in Anim. Breed. Abst., 6, 295.)

SNEDECOR, G. W. (1940): "Statistical Methods," The Iowa State College. Press, Ames, Iowa.

TERRILL, C. E.; and HAZEL, L. N. (1947): Am. J. Vet. Res. 8, 66-72.

VASIN, B. N. (1940): Trud. Inst. Genet. (Mosk.) No. 13: 155-74. (Abstract in Anim. Breed. Abst., 10, 142.)

WEBSTER, W. M. (1937): Proc. VI, Ann. Meet. Sheep Farmers, Massey Agric. College, Palmerston North, pp. 49-55.

OBSERVATIONS ON THE TREATMENT OF *Nosema Apis* WITH DRUGS.

BY

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Summary.

A brief description is given of **Nosema apis** with particular reference to its occurrence in New Zealand. In a series of trials extending over three seasons, atebrin, sulphapyridine, phenamidine, stilbamidine, and pentamidine isethionate were fed in sugar syrup to hives infected with **Nosema apis.** None of these drugs appeared of any value in treating the disease.

INTRODUCTION.

Nosema apis Zander, 1909, is a protozoan parasite of adult honeybees. It is closely related in morphology and life history to *Nosema bombycis* Nägeli, 1857, which gives rise to the destructive silk-worm disease "Pébrine".

Nosema apis was discovered by Dönhoff (1) who first observed its spores in the stomachs of bees in 1857. Although Dönhoff demonstrated that *Nosema apis* spores could transmit the disease he did not discover the parasite in the stage when it has entered the cells lining the bee's stomach. He referred to the disease as "Pilzsucht" (fungus disease). Dönhoff's observations were almost forgotten when Zander, in 1909, found the parasites in the stomach walls of infected bees (2). Zander worked out the complete life history of *Nosema apis* and together with Döflein classified the parasite as a protozoan belonging to the Microsporidia and to the genus *Nosema.* Zander named the parasite *Nosema apis.*

LIFE HISTORY.

Nosema apis causes an infectious disease of adult honeybees. When *Nosema apis* spores reach the stomach of a bee they shed their coats and liberate the parasites which enter the cells lining the stomach. There they grow and multiply rapidly, producing numerous spores which pass through the bee and can infect a fresh host. Field bees become weakened by the enormous number of parasites in the cells lining their stomachs and are often unable to return to the hives from foraging flights. In severe cases bees may crawl from the hives to die or may even fall from the air during flight. In a badly diseased hive all the adult bees may show some degree of infection. Queen bees and drones, as well as worker bees may be attacked but brood is immune. The disease usually reaches its height in the spring, although it may persist throughout the year.

DISTRIBUTION.

Nosema apis has been reported in Germany, Switzerland, Holland, England, Brazil, Canada, Australia, U.S.A., and in almost every country where beekeeping is established commercially. There appears little doubt that *Nosema apis* had been associated with bees long before its discovery by Dönhoff.

ECONOMIC IMPORTANCE.

Nosema apis is a far less serious disease than American foulbrood. Strong colonies with a mild infection usually throw off the disease and recover, but a weak colony with a heavy infection may succumb. If the queen bee becomes infected the colony rapidly loses strength and requeening is usually necessary to prevent its loss. White (3) states that the disease is not particularly malignant in character, being in this respect more like sacbrood than the foulbroods. Burnside (4) says that when accompanied by dysentery brought on by long winter confinement, the disease may spread rapidly within infected colonies and result in the death of the hives later in the winter or in the spring; sometimes heavy losses from *Nosema* disease may continue for weeks after the bees have been flying freely and dysentery has subsided. The shortened life of infected bees weakens or kills the colony. In New Zealand dysentery seldom occurs in bees as the mild winters usually allow them to fly sufficiently often on cleansing flights. Root (5) states that *Nosema,* like sacbrood, has become accepted as a troublesome disease of minor importance, minimized by good beekeeping practice. Wedmore (6) writes that *Nosema* is widespread but not as a rule serious in its consequences.

It appears that *Nosema apis* is endemic in the hive bee and is usually of minor importance except when unknown factors cause a severe epidemic.

TREATMENT OF *Nosema apis* WITH DRUGS.

Because *Nosema apis* is widespread and does not cause severe economic loss to apiarists except perhaps during occasional epidemics, the drastic measures generally used to check American foulbrood are not employed in its control.

Nosema is spread by contamination of the bees' drinking water, by bees robbing badly diseased hives, and by the apiarist moving to uninfected hives hive mats or other equipment fouled by diseased bees. When severe outbreaks of *Nosema* occur practical steps can be taken to lessen the spread of infection from these sources. In addition, requeening of badly infected hives, or combining them with a nucleus is usually beneficial, particularly if the queen has contracted *Nosema.* However, it is difficult to gauge the effectiveness of such control measures, and many investigators have attempted to develop more direct methods of treating the disease in its epidemic form by feeding drugs dissolved in sugar syrup to the infected bees.

White (3) in 1919 carried out experiments to obtain data relative to the effect of β-naphthol, salol (Phenyl salicylate), phenol, salicyclic acid, formic acid, oil of eucalyptus, and bisulphate of quinine on *Nosema* infection. β-naphthol and salol appeared the most effective drugs tried, but White considered his experiments too few for definite conclusions to be drawn as to the extent of their action. Borchert and Polzin (7) in 1933 announced that a 1.25 per cent solution of commercial formaldehyde might be a possible remedy administered to bees in sugar syrup. Falkner (8) in

1939 recommended the treatment of *Nosema* by feeding colloidal sulphur to the bees in sugar syrup. Alpatov and Gubin (9) tried the effect of β-naphthol, xerophorm, picric acid, urotropin, phenol, collargol, and salol on *Nosema*. None of these drugs appeared of value in treating the disease. Gooding and Martin (10) claimed that sulphapyridine fed in syrup would eradicate *Nosema* from a colony. The dose rate was not given. Ashby (11) reported that gramicidin had been used in Russia for treating *Nosema*. The report of the Chief of the Bureau of Entomology and Plant Quarantine (U.S. Department of Agriculture) stated (12) that sulphadiazine and sulphathiazole were not effective against *Nosema apis*. Euflavine (13) has been reported as a promising remedy for *Nosema* but was a little too toxic to the bees. Carbarsone (14), an arsenic compound, has been tested for *Nosema* control in the United States but does not appear satisfactory as in concentrations necessary to arrest the disease it shortens the life of the bee. Gontarski and Seek (15) investigated the action of hydrogen peroxide on *Nosema* but although it seemed promising it has not yet proved possible to cure *Nosema apis* by this method. See Table I.

TABLE I. PREVIOUS TRIALS OF DRUGS FOR TREATMENT OF *NOSEMA*.

Author.	Drugs Tested.	Remarks.
White, G. F. (1919)	β-naphthol, salol, phenol, salicylic acid, formic acid, oil of eucalyptus, bisulphate of quinine.	β-naphthol and salol appeared the most promising.
Borchert and Bolzin (1933)	1.25% solution commercial formaldehyde.	Suggested as a remedy.
Falkner, H. J. (1939)	Colloidal sulphur.	Recommended as a treatment.
Alpatov, V. V. and Gubin, A. F. (1942)	β-naphthol, Xerophorm, picric acid, urotropin, phenol, collargol, salol.	None of these drugs appeared of value.
Gooding, S. and Martin, E. B. (1946)	Sulphapyridine.	Claimed as a cure. No dose rate given.
Ashby, E. (1946) reported.	Gramicidin.	No results available.
Report U.S. Dept. Agr. (1947)	Sulphadiazine and sulphathiazole.	Not effective.
Reference in Bee World, 28, 66. (1947)	Euflavine.	A promising remedy but a little too toxic to the bees.
Reference in Gleanings in Bee Culture, 76, 77. (1948).	Carbarsone.	Unsatisfactory as in the necessary concentrations it shortens life of bees.
Gontarski, H. and Seek, P. (1948)	Hydrogen peroxide.	Appears promising but it has not yet been possible to use it to cure Nosema.

It appears that no satisfactory treatment for *Nosema* depending on the use of drugs has yet been evolved, and the position at present is aptly summed up in the words of Manley (16), "the best way to treat this disease is to keep it at bay if possible, and to do this stocks should be kept strong and well found at all times of the year, which will give them, in all probability, vitality and resisting power enough to prevent the parasite from passing from the endemic phase to the epidemic".

Nosema apis IN NEW ZEALAND.

During the early spring of 1946 many cases of "spring dwindling", or loss of hive strength without apparent cause, were observed in the Hawke's Bay area of the North Island of New Zealand. Examination of samples of bees from many hives showing these symptons led to the discovery that in all cases the bees were infected with *Nosema apis*. Spring dwindling was later reported on a Dominion-wide scale in the spring and summer of the 1946-1947 season, and a survey conducted throughout the Dominion established *Nosema apis* as the causative organism in all cases investigated (17). Although no account of *Nosema apis* in New Zealand had previously been published there is little doubt from past accounts of spring dwindling that *Nosema* has been endemic for many years and has on occasions suddenly flared up to reach epidemic proportions.

During the epidemic several commercial beekeepers suffered severe economic loss because the field bees were often so heavily depleted that little surplus honey could be collected during the main honey flow. The complete loss of several colonies in an apiary was a common occurrence.

Since the 1946-1947 epidemic, *Nosema* has reverted to the more usual endemic state, and no large scale epidemic has occurred, although limited outbreaks are occasionally reported. These are confined at most to a few apiaries in a large district.

TREATMENT OF *Nosema apis* IN NEW ZEALAND.

It was decided to carry out experiments with drugs in an attempt to find a successful method of treating *Nosema apis*.

Trials with Atebrin conducted at Ngatarawa.

Atebrin is a highly effective remedy and prophylactic for malaria. The malarial parasite and *Nosema apis* are both members of the Sporozoa and hence it was considered that atebrin was worth trial as a cure for *Nosema*. A further point in favour of the drug was its cheapness.

Preliminary tolerance tests at Wallaceville established that up to 3 g of atebrin could be fed in one dose to hives in sugar syrup without harm to bees or brood. In the field experiments sufficient 0.1 g atebrin tablets for the required dosage were dissolved in one quart of syrup (2 parts water plus 1 part sugar) which was fed

to the hive in a feeder. Feeding was discontinued when the honey flow commenced. Surplus honey was removed from the hives before feeding the medicated syrup.

The effect of atebrin on *Nosema* was studied by examination of samples of live field bees removed from the hives and forwarded to Wallaceville in queen-cages. On arrival the bees were killed and the stomach contents of individual bees dissected out on microscope slides, where they were fixed after mixing with 1 per cent nigrosin, which served as a background stain. These smears were examined for *Nosema* spores with a microscope. Ten bees were examined from each queen-cage, the number of bees infected with *Nosema* being expressed as a percentage. If spores could not be found in any of the ten smears from individual bees, the abdomens of the bees remaining in the queen-cage were removed and ground up in 12.5 ml of distilled water which was filtered through wire gauze to remove large particles. The suspension was then centrifuged and the deposit examined for spores. This test was much more delicate than the other. In the tables which follow, the results of this method are given when individual dissection of ten bees showed no evidence of *Nosema*. Its adoption is indicated by a figure in brackets which gives the number of bees so treated.

In the first series of experiments 0.5 g of atebrin was fed to hives No. 1, 2, 3 on 16th, 21st and 29th October, 1946, giving a total of 1.5 g per hive. In the second series of experiments 3 g of atebrin was fed to hives No. 4, 5, 6 in one dose.

The control sample was a composite one made up on each occasion from the same four hives at the ends of the rows of hives in the apiary. See Tables II and III.

TABLE II. EFFECT OF FEEDING ATEBRIN TO HIVES IN THREE DOSES EACH OF 0.5.G.

Hive No.	Extent of Nosema apis found from examination of bees on dates indicated								
	21/10/46 (%)	21/10/46 (No.)	29/10/46 (%)	29/10/46 (No.)	6/11/46 (%)	18/11/46 (%)	11/12/46 (%)	7/1/47 (No.)	10/2/47 (No.)	17/3/47 (No.)
1	100			(48)+	30	20	70	(33)-		
2		(42)+	50		20	70	50	(25)-		
3	80			(39)+	40	100	80	(30)+	(30)+	(27)+
Composite Control Sample	100			(83)+	10	80	80	(30)-	(30)+	(27)+

NOTE: Throughout these tables, the figures given in brackets indicate the number of bees included in the composite sample examined after dissection of individual bees had not revealed presence of Nosema apis spores. + sign indicates presence and - sign absence of Nosema apis spores in composite sample.

In the above experiment, on 6/5/47, 27 bees were taken from each hive and the composite sample was centrifuged with negative results except in the case of Hive No. 1 which gave a positive result.

TABLE III. EFFECT OF FEEDING ATEBRIN TO HIVES IN ONE DOSE OF 3.G. ON 18/11/46

Hive No.	Extent of Nosema apis found from examination of bees on dates indicated	
	18/11/46 (%)	11/12/46 (%)
4	10	100
5	100	10
6	100	90
Composite Control Sample	80	80

NOTE On 7/1/47 30 bees, and on 6/5/47, 27 bees from each hive and from the composite control sample were centrifuged with negative results.

Trials with atebrin conducted at Omakau and Maramarua.

In a less detailed experiment in the South Island carried out in conjunction with Mr W. J. Lennon at Omakau five hives infected with *Nosema* were each fed 6 g of atebrin, the drug being given in two doses, each of 3 g. In addition two infected hives were each given 3 g of atebrin. Samples of bees were collected at intervals from these hives and a control hive, and examined at Wallaceville as in the other experiments. Atebrin appeared to have no beneficial effect on the infected hives.

A single hive heavily infected with *Nosema,* situated at Maramarua, was given one dose of 1.5 g atebrin. The course of the disease was compared with that in two control hives by the usual method. The atebrin-treated hive showed no improvement compared with the controls, one of which, indeed, recovered much faster. The field work in this trial was carried out by Mr C. R. Paterson, Apiary Instructor, Hamilton.

From these results no evidence can be adduced that atebrin in the concentrations used was effective in treating *Nosema apis.* Higher concentrations are not readily accepted by bees.

Trials with Sulphapyridine Conducted at Ngatarawa

The same method as before was employed for testing sulphapyridine as a treatment for *Nosema.* Tolerance tests established that 5 g, fed in sugar syrup, did not harm bees or brood. "Soluble"

sulphapyridine (M and B. 693 Soluble) was used, and the trials were carried out in the same apiary as were the atebrin experiments, with the same controls. See Table IV.

TABLE IV. EFFECT OF FEEDING SULPHAPYRIDINE TO HIVES IN ONE DOSE OF 5 G. ON 18/11/46

Hive No.	Extent of *Nosema apis* found from examination of bees on dates indicated				
	18/11/46 (%)	11/12/46 (%)	7/1/47 (No.)	10/2/47 (No.)	17/3/47 (No.)
A	90	40	(30)+	(30)+	(27)+
B	30	100	(25)-		
C	100	30	(28)+	(30)+	(27)-
Composite Control Sample.	80	80	(30)-	(30)+	(27)+

NOTE: On 6/5/47 27 bees from each hive and the composite control sample were centrigued with negative results.

Results obtained would indicate that sulphapyridine in 5 g doses is ineffective in controlling *Nosema apis*.

Trials with Phenamidine Conducted in Hastings

Phenamidine [4:4' diamidinodiphenylether di-(β-hydroxyethanesulphonate)] has been used successfully in the treatment of red-water fever in cattle. This disease is caused by a protozoan parasite, *B. bigemina*, which is a member of the sporozoa. As *Nosema apis* also belongs to the sporozoa it was thought possible that phenamidine might be of use in treating it.

The usual method was employed for testing the value of phenamidine in treating *Nosema*.. Tolerance tests established that a 10 ml ampoule containing 4 g of the drug could be fed in sugar syrup in one dose to a hive without apparent harm to bees or brood.

Difficulty had been experienced in determining the speed with which the medicated syrup was consumed by the bees. During the remaining trials this was overcome by colouring the syrup fed with a dye. A suspension of toluidine red was used for this purpose, viz. 40 g toluidine red plus 1 g saponin in 1 litre distilled water.

The first trials with phenamidine were conducted in Hastings and commenced on 1st August, 1947, when a single dose of 4 g of the drug was fed to selected hives. In spite of the drug two of the hives had died by 17th September, 1947. See Table V.

TABLE V. EFFECT OF FEEDING PHENAMIDINE TO HIVES IN ONE DOSE OF 4 G. ON 1/8/47 AND 5/7/48

Hive No.	Extent of Nosema apis found from examination of bees on dates indicated.											
	1/8/47 (%)	1/8/47 (No.)	14/8/47 (%)	14/8/47 (No.)	17/9/47 (%)	17/9/47 (No.)	5/7/48 (%)	5/7/48 (No.)	19/7/48 (%)	19/7/48 (No.)	16/8/48 (%)	16/8/48 (No.)
1	40				30		.		.		.	
2		(57)+	40		Bees dead		.		.		.	
3	30				50		.		.		.	
4	40			(20)+	30		.		.		.	
5	70					(34)+	.		.		.	
6	50			(17)-		(18)-	40		30		Bees dead	
7	10				30		50		60		"	
8	40					(16)-		(24)+	20		"	
9		(18)+		(27)-	30		40		10		"	
10	60				Bees dead		20		Bees dead			
Control 1	10		20		10		.		.		.	
Control 2	20			(25)-	30		.		.		.	
Control 11	.		.		.		50		Bees dead			
Control 12	.		.		.		30		20		40	
Control 13	.		.		.			(24)+	30		10	
Control 14	.		.		.			(24)+	10			(35)+
Control 15	.		.		.		30		10		Bees dead	

Trials with Phenamidine Conducted in Havelock North

Trials were continued with phenamidine in 1948 when hives in a Havelock North apiary were again fed a single dose of 4 g on 5th July, 1948. By 16th August, 1948, all the bees in the treated hives had died. See Table V.

It is apparent from trials D and E that phenamidine in the dosage used has no beneficial effect on bees infected with *Nosema.*

Trials with Stilbamidine Conducted in Hastings

Stilbamidine (4:4' diamidinostilbene isethionate) has proved effective in the treatment of the protozoan diseases Sudan and Indian kala-azar. Accordingly it was considered worth trying as a cure for *Nosema apis.*

Tolerance tests established that a single dose of 0.75 g of stilbamidine could be fed in sugar syrup to a hive without harm to bees or brood.

The first tests of the drug were made in Hastings on 1st August, 1947, when two hives were each given 0.75 g.

The drug appeared to have no beneficial effect. See Table VI.

TABLE VI. EFFECT OF FEEDING STILBAMIDINE TO HIVES IN ONE DOSE OF 0.75 G. ON 1/8/47 AND 5/7/48

Hive No.	Extent of Nosema apis found from examination of bees on dates indicated.					
	1/8/47		14/8/47		17/9/47	
	(%)	(No.)	(%)	(No.)	(%)	(No.)
11	30		20		30	
12		(32)+		(33)+		(19)+
Control 1	10		20		10	
Control 2	20			(25)-	30	
Hive No.	**Extent of Nosema apis found from examination of bees on dates indicated.**					
	5/7/48		19/7/48		16/8/48	
	(%)	(No.)	(%)		(%)	(No.)
1	30		40		20	
2	20		30		60	
3	10		20		Bees dead	
4	10		50		30	
5		(24)+	10		50	
Control 11	50		Bees dead			
Control 12	30		20		40	
Control 13		(24)+	30		10	
Control 14		(24)+	10			(35)+
Control 15	30		10		Bees dead	

NOTE: When examined on 16/9/48, all bees in Hives Nos. 1,2,4 and 5 were found to be dead.

Trials with Stilbamidine Conducted in Havelock North.

In 1948 trials with stilbamidine were again carried out in a Havelock North apiary.

All the treated hives died, together with two of the controls. See Table VI. The drug appears to have no beneficial effect in the doses given.

Trials with Pentamidine Isethionate.

Pentamidine isethionate has been used for the treatment of trypanosomiasis and kala-azar. It was considered possible that this substance might be of value in the treatment of *Nosema apis*.

Tolerance tests established that a single dose of 0.25 g of the drug could be fed in sugar syrup to a hive without harm to bees or brood.

Tests were carried out in Havelock North where the drug was fed in one dose of 0.25 g to each of four hives on 16th September, 1948. See Table VII.

TABLE VII. EFFECT OF FEEDING PENTAMIDINE ISETHIONATE TO HIVES IN ONE DOSE OF 0.25 G. ON 16/9/48

Hive No.	Extent of *Nosema apis* found from examination of bees on dates indicated			
	16/9/48 (%)	5/10/48 (%)	(No.)	26/10/48 (%)
A	40	50		40
B	40	10		70
C	60		(25) +	50
D	70	10		30
Control E	30	30		90
Control F	60	10		20

Colonies infected with *Nosema apis* appeared to derive no benefit from the drug.

DISCUSSION.

In the seasons following that of 1946-1947, when the epidemic of *Nosema apis* occurred, the disease reverted to its more usual endemic form, and difficulty was experienced in obtaining cases suitable for treatment with drugs. Throughout the trials every care was taken to pick control hives with approximately the same degree of infection as those treated with drugs.

When considering the results of the trials it should be noted that laboratory checks on samples of bees removed from hives treated with drugs were supplemented by careful field observations of the strength and general activity of both treated hives and controls. The field observations were continued for months after sampling of hives had ceased. In all cases field observations confirmed the results of laboratory examinations.

Gooding and Martin (10) claimed that *Nosema* could be eradicated from an infected colony by feeding sulphapyridine in sugar syrup. However, they give no details regarding dosage so that a close comparison with the results of trials described in this paper is not possible.

It appears that atebrin, sulphapyridine, phenamidine, stilbamidine, and pentamidine isethionate, in the strengths fed are of no value in treating *Nosema apis*.

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REFERENCES.

(1) DÖNHOFF and LEÜCHART (1857): Bienen-Zeitung., 13, 66-67, 72. 13, Nr. 6, p. 66-7, 72, March 30.
(2) ZANDER, E. (1909): Leipziger Bienen-Zeitung, 24, 147-50, 164-6.
(3) WHITE, G. F. (1919): U.S. Dept. Agr. Bull. No. 780.
(4) BURNSIDE, C. E. (1936): U.S. Dept. Agr. Circ. No. 392.
(5) ROOT, A. I. (1945): "The ABC and XYZ of Bee Culture", A. I. Root Co., Medina, Ohio, U.S.A. p.486.
(6) WEDMORE, E. B. (1946): "A Manual of Bee-keeping", Edward Arnold & Co., London, p.369.
(7) BORCHERT and POLZIN (1941): Bee World, 22, 78.
(8) FALKNER, H. J. (1939): Ibid, 20, 72.
(9) ALPATOV, V. V.; and GUBIN, A. F. (1942): Zoologicheskii Zhurnal, 21, 147-52. (Abstract in Biol. Abstr. 20, 9183.)
(10) GOODING, S.; and MARTIN, E. B. (1946): Bee Craft, 28, 66.
(11) ASHBY, E. (1946): Bee World, 27, 90.
„ „ (1947): Ibid, 28, 52.
„ „ (1947): Ibid, 28, 66.
(14) „ „ (1948): Gleanings in Bee Culture, 76, 77.
(15) GONTARSKI, H.; and SEEK, P. (1948): Bee World, 29, 93.
(16) MANLEY, R. O. B. (1948): "Honey Production in the British Isles". Faber and Faber Ltd., London, p.320.
(17) PALMER-JONES, T. (1947): N.Z.J. Agr., 74, 48.

SPRAY INJURY FROM USE OF LEAD ARSENATE ON APPLE TREES.

BY

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Summary.

The occurrence on apple trees of fruit and foliage injury resulting from use of sprays containing lead arsenate is discussed. Evidence from field trials shows that:—

(1) Lead arsenate mixed in artesian water (temporary hardness 7.0 grains per gallon) can cause severe foliage injury and slight to moderate fruit injury on apple trees of the Sturmer variety in the Hawkes Bay district.

(2) Addition of 3 lb hydrated lime to 100 gal spray containing 1½ lb lead arsenate reduces, but does not prevent, foliage damage.

(3) When lime sulphur plus colloidal sulphur is combined with lead arsenate plus hydrated lime injury is negligible.

(4) If colloidal sulphur is omitted from the complete mixture of (3) above, foliage damage becomes very severe.

(5) Increase in concentration of lime sulphur increases the severity of leaf damage.

(6) Fruit injury increases with leaf damage but not in the same proportion.

(7) Addition of the wetting agent, Agral 2, to lead arsenate plus lime sulphur and colloidal sulphur causes a characteristic type of injury on fruit. Inclusion of hydrated lime in the mixture reduces the severity of injury which then shows as fruit russet.

From comparison of the above results with laboratory analyses of spray mixtures containing lead arsenate it is concluded that:—

(8) Development of arsenic damage on trees is not directly correlated with occurrence of soluble arsenic in spray mixtures.

(9) Chemical reactions in spray residues on trees are of major importance in causing arsenic injury.

INTRODUCTION.

In apple orchards of New Zealand acid lead arsenate is widely used for control of chewing insects. It is applied at dosages of 1½ to 2 lb to 100 gal alone or in combination with sulphur and polysulphide sprays. Injury to fruit and foliage not uncommonly follows the use of these mixtures and at times is of serious proportions. The more important factors contributing towards tree-damage are discussed in the present paper.

SYMPTOMS AND INCIDENCE OF ARSENIC INJURY.

Soluble arsenic is known to be toxic to plants. It has been shown that the principal cause of plant injury following the use of lead arsenate is due to the production of soluble compounds of

arsenic. As demonstrated by Swingle, Morris, and Burke (1923), and supported by experience in New Zealand, damage is more severe on foliage than on fruit. Older leaves are more subject to damage than younger leaves and consequently injury becomes accentuated in the late summer.

Symptoms of arsenical injury on leaves are diffiicult to distinguish from injury caused by nutritional disorders, adverse weather conditions, and other spray materials. Purple colouration of veins and leaf tissues is followed by necrosis, frequently around leaf margins and extending towards the mid-rib. Necrotic tissues are brown in colour and often exhibit a dark and light brown zonation (Fig. 1). On fruit, a smooth light brown russet sometimes occurs.

Fig. 1.—Arsenic injury on apple leaf of the sturmer variety.

Of the main commercial varieties, Sturmer, Cox's Orange Pippin, and Dunn's Favourite are most subject to damage.

A distinctive type of fruit injury also occurs where the wetting agent, Agral 2 is used in combination with lead arsenate. Typically this consists of "water soaked" tissue, slightly sunken and accompanied by russetting. Damage often occurs as circular or crescent shaped bands, irregular in outline and located on the under surface of fruits. The degree of injury varies from slight russetting to the type shown in Fig. 2.

Fig. 2.—Typical "Agral" injury on Sturmer apple caused by combined sprays of Agral 2 and lead arsenate.

FACTORS RESPONSIBLE FOR THE FORMATION OF SOLUBLE ARSENIC.

Hardness of Spraying Water.

De Ong (1921) pointed out that foliage damage following use of lead arsenate was frequently associated with hardness of spray water. By laboratory analysis Ginsburg (1937) showed that waters of equal hardness reacted differently with lead arsenate, those high in carbonates and bi-carbonates being principally responsible for production of soluble arsenic. In New Zealand, Clark (1938) found that waters high in temporary hardness (as determined by titration with standard acid) caused increased production of soluble arsenic. Where content of sodium carbonate was high there was similarly a marked increase in soluble arsenic as shown in Table I. In later analyses, results of which have not been published, Clark showed that soluble arsenic was not increased when lead arsenate was suspended in distilled water made hard with calcium bi-carbonate*.

*Temporary hardness was obtained by passing carbon dioxide into distilled water containing sufficient calcium hydroxide to give a hardness of 6.0 grains per gal.

This result does not necessarily contradict the general finding of Ginsburg (1937) since his work did not differentiate between carbonates and bi-carbonate of calcium and of other metals. It seems probable, therefore, that constituents of hard water other than calcium bi-carbonate are responsible for production of soluble arsenic.

TABLE I. PERCENTAGES OF LEAD ARSENATE IN SOLUTION AS SOLUBLE ARSENICAL COMPOUNDS FROM LEAD ARSENATE ALONE AND IN COMBINATION WITH VARIOUS SPRAY MIXTURES AND WATERS OF DIFFERENT DEGREES OF HARDNESS

[1] Spray mixture, quantities to 100 gal water.	Percentage solubility of lead arsenate.
Distilled water	
L/A 1½ lb	0.11
L/A 1½ lb + Agral 2 2 oz	0.11
" + " 4 oz	0.11
" + H/L 3 lb	0.023
" + Agral 2, 2 oz + H/L 3 lb	0.007
Distilled water + L/S 5 pt	
L/A 1½ lb	11.5
" + H/L 3 lb	0.015
Distilled water + C/S 2 lb	
L/A 1½ lb	0.05
" + H/L 3 lb	0.015
Distilled water + L/S 5 pt + C/S 2 lb	
L/A 1½ lb	11.5
" + Agral 2 2 oz	11.5
" + H/L 3 lb	0.02
" Agral 2 2 oz + H/L 3 lb	0.01
[2] Hastings water	
L/A 1½ lb	2.3
" + Agral 2 2 oz	1.5
" + " + H/L 3 lb	0.08
[3] Foxton water	
L/A 1½ lb	3.1
" H/L 3 lb	0.08

1. L/S = Lime sulphur, 16.0% polysulphides; L/A = Acid lead arsenate; C/S = Colloidal sulphur paste, 50 sulphur; H/L = Hydrated lime. Agral 2 = Proprietary wetting agent.
2. Temporary hardness 6.5 grains per gal.
3. Temporary hardness 16.0 grains per gal and sodium carbonate 7 grains per gal.

Use of Lead Arsenate With Lime Sulphur.

The mixture of lead arsenate with lime sulphur leads to chemical reactions resulting in production of soluble arsenical compounds. Goodwin and Martin (1925a) showed that in distilled water and in the presence of oxygen, production of soluble arsenic was negligible, whereas considerable increase occurred when carbon dioxide was

passed through the mixture of lead arsenate and lime sulphur. They demonstrated that addition of hydrated lime to the mixture reduced soluble arsenic. Hodgkiss, Frear and Worthley (1938) also found increased amounts of soluble arsenic from combination of lead arsenate and lime sulphur, the production of soluble arsenic increasing as concentration of lime sulphur was reduced. They also confirmed the finding of Goodwin and Martin (1925b) that the addition of hydrated lime to the combined sprays reduced the amount of arsenic in solution. With a mixture of 3 lb lead arsenate and 2 gal lime sulphur to 100 gal water it was found that soluble arsenic was progressively reduced with increase of hydrated lime up to 3 lb. Clark (1938) showed the presence of greater quantities of soluble arsenic from mixtures of lime sulphur and lead arsenate than from any other combination. He found that hydrated lime reduced the amount of arsenic in solution to negligible amounts (see Table I).

Use of Lead Arsenate With Wetting Agents.

It has been generally assumed in New Zealand that the use of soluble soaps in combination with lead arsenate, lead to increase in soluble arsenic. Ginsburg (1937) demonstrated, however, that this was true only where soft waters were used and that with hard waters the use of soluble soaps resulted in reduction of soluble arsenic.

In the 1936/37 season the proprietary wetting agent, Agral 2, was used for the first time by orchardists in the Nelson Province. Many growers reported fruit and foliage injury and in all cases it was found that Agral 2 had been used in combination with lead arsenate but without the addition of hydrated lime. Where hydrated lime was used, damage was negligible or absent. Laboratory analyses (Clark, 1938) showed no increase in production of soluble arsenic from the addition of Agral 2 (see Table I).

FIELD TRIALS IN NEW ZEALAND.

Field trials were carried out during the period 1939 to 1947 in an attempt to correlate the general findings discussed above with the occurrence of tree injury in the field. At the time trials were commenced, growers were recommended to use hydrated lime at twice the weight of lead arsenate wherever sprays were combined with lime sulphur. When lead arsenate was used alone, hydrated lime was considered unnecessary, since practically all acid lead arsenates in use had low water solubilities, conforming to the following standards recommended by Cunningham and Cottier (1933):

> Ratio of lead oxide (PbO) to arsenic pentoxide (As_2O_5), 2:1; Minimum content of arsenic pentoxide, 32 per cent; Water-soluble arsenic pentoxide, less than 0.2 per cent; Particle size, 90 per cent less than 10μ.

Most of the arsenates in use in New Zealand have water solubilities considerably below 0.2 per cent when measured in distilled water. In orchard practice, waters of varying degrees of hardness are used, and although laboratory analyses showed that increased solubility could be avoided by use of hydrated lime, field observation suggested that tree injury might still occur. It had also been observed that where colloidal sulphur was used in the spray mixture tree injury appeared to be reduced, an observation which was not explicable from laboratory analyses (see Table I). Field experience from the use of Agral 2 wetting agent was also at variance with measurements of water soluble arsenic.

Treatments were carried out in the Hawkes Bay district on the Sturmer variety. Spray mixtures were prepared with artesian water having a temporary hardness of 7.0 grains per gallon. Applications of experimental sprays were commenced at petal-fall and repeated at intervals of 14 to 18 days extending to 21 days after mid-summer. Thus seven or eight applications were made through the season. All treatments were based on various mixtures of the following materials at dilutions used in standard commercial spray programmes:

> Lime sulphur, 15 per cent polysulphides, w/w; Colloidal sulphur, 40 per cent sulphur paste, 90 per cent particles less than 1μ; Acid lead arsenate powder (as described above); Hydrated lime, 95 per cent calcium hydroxide; Agral 2, proprietary wetting agent containing alkylated naphthalene sulphonates.

Treatment effects were recorded by grading trees at full foliage according to degree of leaf injury. For this purpose trees were graded from 1 to 10, grade 1 being free of damage and grade 10 the most severe injury showing in any series of treatments. Because of differences in degree of damage due to variable weather conditions from one season to another and the difficulty of maintaining a standard for grading after such a time interval, no attempt has been made to correlate degree of injury between different seasons. Records of fruit injury were based on commercial standards of russet, or where Agral 2 was used, on percentage fruits showing typical "Agral" injury.

Experiment 1—Season 1940/41.

Results given in Table II show that hydrated lime caused a marked reduction in foliage injury although degree of injury still remained higher than in the check treatment. Fruit injury as shown by russet gradings was increased by all spray treatments as compared with check and with the treatment in which hydrated lime was used alone. In Treatments 2 and 3 (hydrated lime omitted from combined sprays of lead arsenate, lime sulphur and colloidal sulphur) the degree of russet was not as great as would be expected from the severity of foliage injury.

TABLE II. FRUIT RUSSET AND DEGREE OF FOLIAGE INJURY FROM SPRAY PROGRAMMES WITH AND WITHOUT HYDRATED LIME (SEASON 1940/41 ON STURMER APPLE TREES IN HAWKES BAY)

Treatments* (seven applications during season)	Percentage fruits[x] showing more than 15% russet	Degree[+] foliage injury
1. L/S ½ gal + C/S 1 lb + L/A 1½ lb + H/L 3 lb	15.0	4
2. L/S ½ gal + C/S 1 lb + L/A 1½ lb	31.3	10
3. " " + L/A 1 lb + H/L 3 lb	15.2	4
4. L/S ½ gal + C/S 1 lb + L/A 1 lb	17.3	10
5. - - - - - - - - - - - - - - - - + H/L 3 lb	4.3	2
6. Check - no sprays	8.5	2

* All dilutions made to 100 gal water.
x Mean percentage from six trees.
\+ Based on grading of foliage injury from 1 = no damage, to 10 = very severe damage with approximately 25 per cent defoliation.

TABLE III. DEGREE OF FOLIAGE INJURY FROM COMBINATION OF THE DIFFERENT MATERIALS USED IN THE GENERAL SPRAY PROGRAMME. (SEASON 1942/43 ON STURMER APPLE TREES IN HAWKES BAY)

Treatments* (seven applications during season)	Degree foliage injury
1. L/S ½ gal + C/S 1 lb + L/A 1½ lb + H/L 3 lb	3½
2. - - - - - " " "	2½
3. - - - - - - - - - - " "	4
4. - - - - - - - - - - " + H/L 1 lb	7
5. - - - - - - - - - - "	10
6. Check - no sprays	2

* All dilutions made to 100 gal water.

Experiment 2—Season 1942/43.

Table III shows that foliage damage was very severe where lead arsenate was used alone, and while progressively reduced by the addition of 1 to 3 lb of hydrated lime, still remained greater than on check treatments. Where colloidal sulphur was included in the mixture, the degree of foliage injury was still further reduced, being only slightly higher than the check. Addition of lime sulphur caused a slight increase in damage.

Records of fruit injury were not taken but from observation it was apparent that no marked differences in degree of russet occurred between treatments.

Experiment 3—Season 1943/44.

Experiment 2 was repeated in the modified form shown in Table IV. Lead arsenate used alone caused more injury than lead arsenate plus colloidal sulphur and lime sulphur. The addition of hydrated lime reduced but did not prevent foliage damage. Where lime sulphur plus colloidal sulphur was used alone, leaf injury was

only slightly greater than on the check. Although fruit injury increased with degree of foliage injury they did not appear to be directly correlated. Thus where lime sulphur plus colloidal sulphur was used alone fruit damage was higher than might have been expected, whereas the use of lead arsenate alone caused only a moderate increase in fruit russet in spite of the severity of foliage injury.

TABLE IV. DEGREE OF FRUIT RUSSET AND FOLIAGE INJURY FROM USE OF DIFFERENT COMBINATIONS OF MATERIALS USED IN THE GENERAL SPRAY PROGRAMME. (SEASON 1943/44 ON STURMER APPLE TREES IN HAWKES BAY)

Treatments* (seven applications during season)	Percentage fruits[x] showing more than 15% russet	Degree foliage injury
1. L/S + gal + C/S 1 lb + L/A 1½ lb + H/L 3 lb	17.4	3
2. L/S + gal + C/S 1 lb + L/A 1½ lb	23.7	8
3. - - - - - - - - - - " "	19.3	5
4. - - - - - - - - - - "	29.6	10
5. L/S ½ gal + C/S 1 lb - - - - - -	13.6	2.5
6. Check - no spray	7.0	2

* All dilutions made to 100 gal water.

[x] Mean percentage from six trees.

TABLE V. DEGREE OF FOLIAGE INJURY FROM USE OF DIFFERENT MIXTURES OF MATERIALS USED IN THE GENERAL SPRAY PROGRAMME. (SEASON 1946/47 ON STURMER APPLE TREES IN HAWKES BAY)

Treatments* (seven applications during season)	Degree foliage injury
1. L/S ½ gal + L/A 1½ lb + H/L 3 lb	7
2. L/S 1 gal " "	10
3. C/S 1 lb + L/A 1½ lb + H/L 3 lb	3
4. - - - - - - " "	4½
5. Check - no spray	2

*All dilutions made to 100 gal water.

[x]Based on grading of six trees from each treatment for foliage injury.

Experiment 4—Season 1946/47.

In Experiments 2 and 3, lime sulphur was always used in combination with colloidal sulphur. This mixture tended to reduce foliage injury when added to lead arsenate, an effect not generally recognized in practice. In the present experiment treatments were used in which lime sulphur alone was included in a mixture of lead arsenate and hydrated lime. Results given in Table V show that severe foliage damage occurred, and that when the concentration of lime sulphur was increased leaf damage became very severe, trees being practically defoliated. In both treatments the degree of damage was much higher than from lead arsenate plus hydrated lime alone. When colloidal sulphur was added to the latter, foliage injury was reduced to a degree only slightly higher than that on check trees.

Records of fruit russet were not taken because spray injury could not be distinguished from that caused by a frost which had occurred earlier in the season.

Experiment 5—Season 1939/40.

In Table VI are given results of treatments in which Agral 2 was combined with normal spray mixtures with and without the addition of hydrated lime.

The degree of foliage injury was not recorded but it was observed that use of Agral 2 caused only a slight increase in damage.

On fruit severe damage occurred. "Agral" type injury was most severe when hydrated lime was omitted from the combined sprays. In the presence of hydrated lime symptoms of "Agral" injury were almost entirely suppressed but damage showed as typical fruit russet. As a result, the recorded percentage of fruit russet was increased, and exceeded that from the treatment in which hydrated lime was omitted.

In treatments in which Agral 2 was not used, the absence of hydrated lime did not affect the percentage of fruit russet but resulted in development of severe foliage injury as in previous experiments.

TABLE VI. PERCENTAGE FRUIT RUSSET AND "AGRAL" INJURY FROM SPRAY TREATMENTS. (SEASON 1939/40 ON STURMER APPLE TREES IN HAWKES BAY)

Treatments* (seven applications during season)	Percentage fruits[x] showing 15 to 50% russet	Percentage fruits[x] showing more than 50% russet	Percentage fruits[+] "Agral" injury
1. L/S ½ gal + C/S 2 lb + L/A 1½ lb	0.3	0.0	0.0
2. L/S ½ gal + C/S 2 lb + L/A 1½ lb + Agral 2 3 oz	38.3	5.0	45.3
3. L/S ½ gal + C/S 2 lb + L/A 1½ lb + H/L 3 lb	0.7	0.0	0.0
4. L/S ½ gal + C/S 2 lb + L/A 1½ lb + H/L 3 lb + Agral 2 3oz	50.7	14.7	2.7
5. Check - no spray	0.3	0.0	-

* All dilutions made to 100 gal water.

x Mean percentage from three trees.

\+ Mean percentage from all fruits whether russetted or not showing typical "Agral" injury.

DISCUSSION.

From results of these experiments it is apparent that lead arsenate can cause severe injury on apples in the Hawkes Bay district and that foliage is much more subject to damage than fruit. By the addition of 3 lb hydrated lime to each 1½ lb lead arsenate, foliage injury is reduced but not entirely avoided. Where lime sulphur and colloidal sulphur are used in the mixture of hydrated lime and lead arsenate foliage damage is negligible. If, however, colloidal sulphur is omitted from the combined sprays, extensive foliage injury may occur. Increase in the concentration of lime sulphur in the spray mixture appears to increase the severity of foliage damage rather than reduce it, a result in conflict with the findings of Hodgkiss, Frear and Worthley (1938).

When results of these trials are compared with laboratory measurements it is obvious that the degree of tree injury is not in direct relation to the content of soluble arsenic in the spray mixture. Thus, when hydrated lime is added to lead arsenate mixtures in Hastings water (see Table I) soluble arsenic is reduced to negligible amounts. In spite of this, arsenic injury, though reduced, still remains fairly severe. Of greater significance are differences between lead arsenate used alone and lead arsenate combined with lime sulphur and colloidal sulphur. Analyses show that soluble arsenic is much higher in the latter but damage is less. The effect of colloidal sulphur is of considerable interest since from the results given in Table I it does not appear to influence production of soluble arsenic; however, field evidence shows reduction of foliage injury.

Although Agral 2 is not now used in apple spray programmes, results from the trial recorded are of general interest. With this material, damage occurs mainly on fruit, suggesting a major difference in the mechanism of injury (as compared with normal arsenic damage). In the trial recorded (Experiment 5), Agral 2 was not used alone so that the possibility of the wetting agent being responsible for damage cannot be ignored. On the other hand in commercial orchards where Agral 2 has been used in spray mixtures not containing lead arsenate, no cases of injury have been reported. It appears likely, therefore, that the damage resulting from Agral 2 in spray mixtures is due primarily to soluble arsenic with the wetting agent causing increased injury by physical means. Table I shows that Agral 2 does not influence water solubility of lead arsenate. This is of practical significance since analyses of lead arsenate powders for water soluble arsenic might not give a reliable guide to phytocidal effects if small amounts of wetting agent similar to Agral 2 were present in the powders.

Results here discussed lead to the following general conclusions: (1) that laboratory analyses though showing reactions in spray mixtures leading to variation in soluble arsenic give only a partial explanation of the causes of arsenic injury in the field; and (2) that chemical reactions in spray residues on trees (or possibly physical effects where wetting agents similar to Agral 2 are used) are of major importance in the development of arsenic injury.

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REFERENCES.

CLARK, P. J. (1938): N.Z. Orchardist, 11, 156-57.
CUNNINGHAM, G. H.; and COTTIER, W. (1933): N.Z. J. Agr., 46, 211-21.
DE ONG, E. R. (1921): Cal. Agr. Exp. Sta. Bull. 338, 301-14.
GINSBURG, J. M. (1937): J. Econ. Ent., 30, 583-90.
HODGKISS, W. S.; and FREAR, D. E. H.; and WORTHLEY, H. N. (1938) Ibid., 31, 443-55.
GOODWIN, W; and MARTIN, H. (1925a): J. Agr. Sci., 15, 307-26.
" " " " (1925b): Ibid., 15, 476-90.
SWINGLE, D. B.; MORRIS, H. E.; and BURKE, E. (1923): J. Agr. Res., 24, 501-39.

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